



Review

A Review on Bioremediation - An Emerging Technology for Treatment of Radionuclide Waste

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Received: July 15, 2022 Revised: August 16, 2022 Accepted: September 26, 2022 Published: January 16, 2023

Abstract

Radioactive nuclides are utilized in large quantities in different sectors such as nuclear power plants to address energy demand. As a result, there is a massive amount of radioactive waste produced from nuclear sites and its spent sources including mining activities, nuclear weapons recycling, nuclear weapon, and nuclear energy generation. The widespread discharge of radionuclides into the environment and their mobility is a worldwide matter of concern given its health risks to living organisms. Hence, there is a need to embrace sustainable technologies for the effective management of radioactive nuclide wastes. Bioremediation is a promising and eco-friendly approach to remediate radioactive waste. The current review discussed various modes of bioremediation such as microbial, phyto, and myco-remediation for the management of radioactive waste. The interaction of microorganisms with various actinides and their fission products are also discussed. Moreover, a sustainable and efficient strategy for radioactive nuclide waste management in developing countries is also highlighted.

Keywords: radioactive wastes, bioremediation, actinides fission products, radioactive waste management

Citation: Cheema HA. A Review on Bioremediation - An Emerging Technology for Treatment of Radionuclide Waste. *J Mod Agric Biotechnol*, 2023; 2(1): 2. DOI: 10.53964/jmab.2023002.

1 INTRODUCTION

Atoms with sufficient nuclear energy to destabilize them are called radionuclides. This excessive nuclear energy can be released in three different ways including alpha, beta and gamma radiation^[1]. The emission of excessive nuclear energy from nucleus in forms of alpha, beta and gamma radiation is known as radioactive decay/radioactivity^[2]. Both natural and anthropogenic sources originate radiation

in the environment. It is estimated that more than 80% of the worldwide environmental radiation is from natural sources^[3]. Naturally occurring radionuclide can be grouped into three types viz. primordial, secondary radionuclides and cosmogenic radionuclides. Primary radionuclides produce mainly from the interiors of stars as a part of the earth crust, while secondary radionuclides are derived from the decay of primary radionuclides and are radiogenic

isotopes. Primordial radionuclides have longer half-lives in contrast to secondary radionuclides. Cosmic radionuclides are generated after interactions of cosmic rays^[4,5].

It is commonly believed that radionuclides can be generated anthropogenically by nuclear reactors, particle accelerators, or radionuclide generators. They are continuously released into the environment following nuclear power weapon tests, nuclear energy activities, and nuclear power plant accidents^[6-9]. In the previous six decades, the list of generated radioactive nuclide by fission reactors is constantly elongating and includes Neptunium-237, Plutonium-238, Americium-241, Hydrogen-3, Carbon-14, Krypton-85, Strontium-90, Technetium-99, Iodine-129 and Cesium-137 along uranium from different nuclear sites^[10]. Radionuclides have been found in water, soil and air currents around the globe, and they are usually dumped on the ground depending upon their weight. At times, heavy rains may carry the radioactive elements to the ground^[4]. Radioactive nuclide that persists in the soil can dissolve into solution, form complexes with organic matter in the soil, or precipitate out easily. The stasis of these radioactive nuclides in the uppermost layer of the soil is considered detrimental to environment. Radioactive nuclides are found either in soil or sediments in aquatic system where they can be easily incorporated into the food chain prompting health risks^[6,11-14].

Table 1 describes the release of radioactive nuclide waste from nuclear sites and its spent sources including mining activities, industrial activities, land fillings, agrochemical waste, nuclear weapons recycling, nuclear weapon and nuclear energy generation, resulting in severe environmental hazards that cause serious health issue^[15-18]. The discharge of radioactive nuclides to the environment continuously has been increased as nuclear energy demands increases day by day. As a result, the adverse and harmful effects of radionuclide waste on the environment and organisms are increasing. Table 2 summarizes the effect of various radioactive nuclide on biota along with emitted radiation. Hereafter, there is a need to implant an efficient method and strategy for the better management and treatment of radioactive nuclide waste. Currently, the removal of radioactive nuclide from all of the waste streams is a worldwide matter of concern.

Different physio-chemical methods have been introduced for their recovery from waste streams such as liquid-liquid extraction, chemical precipitation, electrochemical processes, coagulation, ion exchange, membrane processes and co-precipitation^[19]. However, these methods have some drawbacks such as their low cost-effectiveness, high degree of selectivity, and the need for optimal environmental conditions to improve their efficiency. Therefore, this review focuses on "Bioremediation" for the recovery of radioactive nuclides from spent nuclear sources. It is a process that

based on metabolic activity of microbes for the removal of radioactive nuclide from the waste. Due to the commercial benefits of microbial systems such as their low costs and easy cultivation without chemical contaminations produced during the biological process, the microbial system has been adapted worldwide for the efficient removal of radioactive waste nuclides^[20]. Therefore, biotechnology performs a significant role in different industrial sectors in favor of environmental protection^[15]. Different research programs have been introduced to access the potential of microbes for the recovery of radioactive nuclide. Table 3 depicts the comparative analysis about advantages of bioremediation with other remediate methods.

2 BIOREMEDIATION OF RADIONUCLIDE

Microbes catalyze the conversion of inorganic and organic material naturally and can be utilized efficiently to solubilize or immobilize various forms of radioactive nuclides and toxic metals that are part of waste streams^[10,21,22]. The basic mechanisms of microbial bioremediation of radionuclides are oxidation-reduction reactions, which affect the formal charges and their solubility, formation of complexes, bioaccumulation and biosorption, as depicts in Figure 1.

Microbial activities are greatly influenced by the capability of electron acceptance and donation^[23-25]. Most microbes utilize oxygen as electron acceptor, while under anaerobic conditions nitrate, sulfate, metals, and carbon dioxide are widely used as exchange sources for electron capturers of microbes^[26]. Under anaerobic conditions, the degree of precipitation and solubilization of radioactive nuclide is enhanced. Effective bioremediation of radioactive nuclides is predicated on the formation of complexes associated with physical, biological and chemical processes. The fundamental mechanisms include dissolution, oxidation, precipitation, reduction, sorption and leaching, and all these processes can reduce the toxicity and transfer the radioactive nuclides in biogeochemical cycle. All these basic mechanisms are realized via microbes, and bioprocess actively involved in the bioremediation of radioactive nuclides are designed to immobile them in consign to accelerate the recovery of radionuclides from waste stream^[27,28]. Turick and Berry^[29] studied microbial activity involved in the degradation of concrete at nuclear waste storage sites. Reena et al.^[30] presented a complete database of bacteria and fungi, both wild kind and recombinant, which might be used in remediation of radioactive nuclide waste and has been created as 'BioRadBase'. Xu and Zhou^[31] noted that the great benefits of using microbes for the remediation of radioactive waste are high specificity, reusability more efficient, cost effective, minimum pollution and proper elimination of pollutant.

3 CLASSIFICATION OF BIOREMEDIATION

Bioremediation is further classified into three types (microbial, fungal and plant-based remediation) based on

Table 1. Various Streams of Radioactive Material Pollution and Various Bioremediation Modes to Manage Pollution ^[15-17]

Sources	Various Steam of Radioactive Material Pollution
Industrial activities	Nuclear weapon recycling, plastics, preservatives, microelectronics and refineries
Mining activities	Smelting, mine spoil, wtailing and metal industries
Waste disposal	Sewage, sludge and leachate from landfills
Agrochemicals	Extensive use of fertilizer and pesticides
Various Bio-remediation Modes	
Bacterial/Fungi	Plant
Bio-reduction	Phyto-extraction
Bio-accumulation	Phyto-stabilization
Bio-mineralization	Rhizo-filtration
Bio-sorption	Phyto-volatilization

Table 2. Various Radioactive Nuclide with Emitted Radiation and Effect on Biota ^[12-14]

Radioactive Nuclide	Half-life	Emitted Radiation (MeV)	Effect on Biota
U ^{238, 235, 234, 239}	69y-109y	α, β (4.2-205)	Dangerous and abundant, contains natural fissile elements as reactant. Absorbed by most cells/organisms
Pu ^{238, 239, 240, 242}	88y-105y	α (0.1-560)	Highly dangerous and accumulates especially in bones. Highly fissile / reactive, sustains chain reaction
Ra ^{226, 228, 223, 224}	11d-103y	α, β (4.9-6.0)	All isotopes are highly radioactive /dangerous. A heavy metal which incorporates easily into cells/tissues
Rn ^{210, 222, 224}	2.4h-3.8d	α, β (2.8-6.0)	Noble gas that has only radioisotopes present. Intensely radioactive and dangerous even from natural sources
Cs ^{134, 137, 133}	30y-106y	β (1.2-2.1)	Biological properties like K, can substitute for K in cells. Highly toxic when released into the environment
Sr ^{89, 90, 86, 88}	50d-29y	β, γ (0.6-1.2)	Properties like Ca and can substitute for Ca in tissues. Often toxic when released into the environment
Np ^{237, 236, 235}	2.5d-106y	α, β (1.0-5.2)	All forms are radioactive. Product of U fission, present in w
Am ^{241, 243, 242}	103y-104y	α (5.2-5.6)	Product of U fission and often present in high levels in industrial waste. Adheres strongly to soil particles
Tc ^{99, 98, 97}	4d-106y	β, γ (0.2-0.8)	All forms are radioactive. Product of U fission, present in waste and dangerous, used in cancer treatment
Po ^{208, 209, 210}	2.8y-138y	α, β (1.4-5.2)	Highly dangerous, is taken-up with no biological function.

Table 3. Comparative Analysis of Bioremediation in Comparison to Conventional Remedial Methods

Advantages of Bioremediation as Compared to Conventional Remedial Methods
Bioremediation takes advantage of the natural ability of microorganisms to extract chemicals from water, soil, and sediment using energy from sunlight.
It is a cost-effective technique compared to other physicochemical treatment methods
Less energy is required as compared to other technologies
Often little to no residual treatment is required
Bioremediation can be done on site and is often less expensive, and site disruption is minimal
Soil stabilization and reduced water leaching and transport of organic compounds in the soil
Typically, lower cost to implement
Enhanced regulatory and public acceptance

the type of biomass, as depicted in [Table 1](#).

3.1 Microbial Remediation

The conversion of the radioactive nuclide into stable

isotopes indirectly by the process of bacterial energy transfer is known as bacterial remediation. Radioactive nuclides are indirectly transformed by reducing and oxidizing agents produced by microbes, which can change

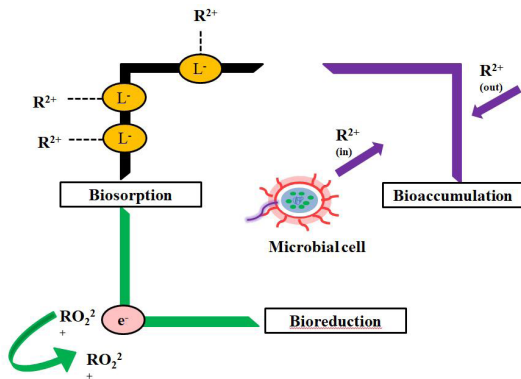


Figure 1. Mechanism of bioremediation of various radioactive nuclear wastes by biologically mediated reduction, sorption and accumulation in living system^[22] (R^{2+} : Radioactive nuclide).

their pH and their oxidation state. Bacterial remediation consists of different processes such as bioreduction, biosorption, bioaccumulations, and biomineralization involved in radionuclide transformation^[16]. Bacteria can easily immobilize the radioactive nuclide by enzymes either directly or indirectly. The basic processes involved are bioreduction, biosorption, biomineralization and bioaccumulation.

3.1.1 Bioreduction

Bioreduction is a process in which bacterial species use redox potential and a reduction occur, through which soluble radionuclide becomes insoluble form. Bioreduction is a reliable technique because it is easy to operate under mild environmental conditions and does not produce hazardous wastes^[17]. Bioreduction is carried out by direct and indirect methods. In the direct enzymatic process, high oxidation state is converted to a less oxidation state by means of anaerobic bacteria. In this process radionuclides are efficiently attached to binding sites on the surface of bacteria and they act as electron acceptor with ethyl lactate as the electron donor during anaerobic respiration. On the other hand, indirect bioreduction involves the indirect reduction of radionuclides to less soluble and less toxic species via sulfate-reducing bacteria. Some common examples of both types of bioreduction bacteria are *Geobacter sulfurreducens*, *Shewanella putrefaciens*, *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*. The basic mechanism of bioreduction for both direct and indirect methods is depicted in Figure 2.

3.1.2 Biosorption

Biosorption, bioaccumulation and biomineralization are similar processes given their direct connection between the cell surface and radioactive nuclide^[32]. All these are active processes dependent on energy transfer systems. Biosorption is a process in which positively charged radionuclides form complexes with normally negatively charged biomass

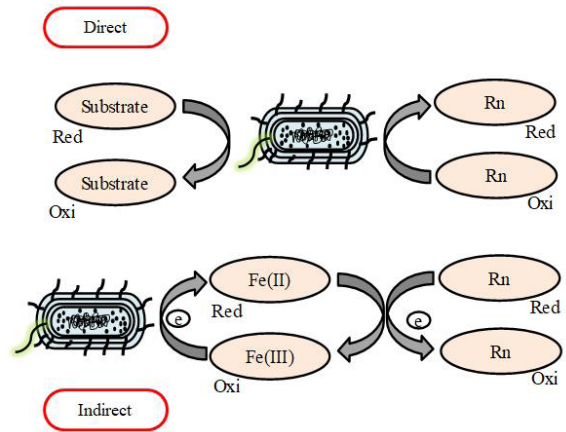


Figure 2. Direct and Indirect enzymatic reduction of radionuclides by bacteria^[22].

either dead or alive^[33]. Biosorption process involves the immobilization of the radioactive nuclide, which is species specific. Factors such as temperature, pH, aeration, growth phase of the cell, secretion of the exopolymer, and composition of the metabolites can affect biosorption. Moreover, the chemical interaction of extracellular biopolymers, electrostatic attraction and functional groups along with metal ions also affect biosorption^[34]. Some bacterial species such as *Citrobacter freundii* and *Firmicutes* have significant capacities for biosorption. The carboxyl group in the cell wall of *Citrobacter freundii* is one of the most active sites of biosorption and has an important effect on sorption^[35]. They also revealed that dead cells display better sorption capability because the whole process was influenced by functional groups other than the biological activity of the cell. The main disadvantage of biosorption is the rapid saturation of nuclide molecules and competitive desorption, which is caused by cations other than the targeted one competing for the cell's binding site^[36]. As a result, biosorption is commonly used to remove low-concentration radionuclides from effluents^[37].

3.1.3 Bioaccumulation

The process of bioaccumulation is defined as the uptake of radioactive nuclide into the cell, where the cell complex is formed by positively charged radioactive nuclide and negatively charged cellular components in the form of small grains or precipitation^[38]. The mechanism of bioaccumulation is based on the formation of a complex in which radioactive nuclides come into direct contact with some ligands such as phosphate, hydroxide, sulphate, forming insoluble substances inside the cell that can be easily removed from the solution. These insoluble forms are less harmful to the environment^[39]. *Micrococcus luteus* is a common example of bioaccumulation of strontium, forming complexes on the cell surface^[17]. The process of bioaccumulation might be active or passive. Active bioaccumulation is a slow and more energy-depleting

process that either depends or not on metabolism, while passive bioaccumulation is more promising due to limited nutrients. In contrast to active process it is a fast and less energy consuming process^[40]. Both intracellular or extracellular accumulation is feasible. Intracellular accumulation of radionuclides is more prominent when existence or non-existence of respective radioactive nuclide influences the permeability of cell membrane^[37].

In *Citrobacter sp.* and *Halomonas sp.*, intracellular accumulation of U⁺⁶ was mostly in the form of phosphates such as hydroxophosphate, polyphosphates, or uranium hydrogen phosphate^[41]. Amachi et al.^[42] showed that the accumulation of iodide on the cell wall of soil *Bacillus subtilis* was shown to increase with the addition of glucose. Ozdemir et al.^[43] studied bioaccumulation of U+6 by *Bacillus vallismortis* and found that after 72h of inoculation, bioaccumulate U+6 was at a concentration of 5mg/L. The amount of U (VI) accumulated was about 50mg/g (metal/dry bacteria), which was later analyzed by UV-Vis spectrophotometry. Another study on radionuclide exchange between uranium and a proton was conducted utilizing a separation factor, and it was shown that Gram-positive bacteria such as *Micrococcus luteus*, *B. subtilis*, and *B. megatarium* could accumulate Th in a solution containing both Thorium and Uranium^[44]. Bioaccumulation is influenced by the molecular properties of the nuclide, as well as the potentials and characteristics of the bacteria present, as it includes rapid interactions with anionic groups in components of the cell surface^[45]. It is also influenced by size and lipid content, with a reduction in size resulting in reduced surface area for accumulation^[46]. The presence of capsules, slime, or S-layers has a significant impact on the bioaccumulation process. Polyphosphate bodies act on the nuclides after their accumulation. Metal tolerance and subsequent radiation tolerance have been linked to intracellular chelation processes^[47]. However, according to Jiang et al.^[48], the surface characteristics of bacterial cell walls determine their adsorption properties. Variations in cell wall composition between Gram-positive and Gram-negative bacteria had little effect on bioaccumulation^[49].

3.1.4 Biomineralization/Bioprecipitation

Biomineralization is the formation of precipitate through the interactions of radioactive nuclide and microbial ligand^[36], which is known as bio precipitation. The precipitation of radionuclides usually occurs in the form of carbonates or hydroxides. The oxidation state and valency of radionuclide have prominent effect on bio precipitation. The product from during biomineralization is a stable composite and biogenic material^[50,51]. In microbial cell, the site of precipitation is known as the ‘nucleation site’ and it depends on the concentration of ligand produced by the cell. The precipitation of radionuclide is influenced by the ligands produced by microbes and biogenic formation of minerals^[36]. Bacterial metabolism and secretions are

prime factor that can change the pH and thus the pH in the vicinity of radionuclides^[37]. Co-precipitation is another phenomenon where elements combine with metal oxide minerals during their precipitation^[38]. *Citrobacter* and *Serratia* is are common example of biomineralization, which release phosphate ligand and form uranyl ions^[52]. Table 4 summarized the mechanisms of radionuclide microbial bioremediation^[53-65]. The use of these mechanisms of microorganisms for radioactive waste depends mainly on the individual capabilities of the species. Therefore, microorganisms appear again and again as they are the best choice for the remediation of radioactive wastes.

3.2 Plant Remediation

The term “phytoremediation” refers to a group of remediation techniques that employ plants to clean or partially clean contaminated sites or reduce the danger of toxins. Phytoremediation is also known as green remediation, botano-remediation, agro-remediation, and vegetative remediation^[66,67]. Plants have the capability to uptake pollutants in the environment through the root system that provides a larger surface area, ease mobilization, and detoxification of contaminants within plants by using a variety of mechanisms such as elimination, containment, and degradation. Such plant characteristics have been employed to effectively remove radioactive waste. The microbial population associated with the plant and their interactions play a crucial role in maintaining the health of the plant. These interactions inhibit phytopathogens by releasing compounds that promote growth, increase the availability of nutrients, encourage detoxification (e.g., degradation, sequestration, and volatilization of pollutants), and enhance stress tolerance by introducing systematically acquired host resistance. Leaves, stems, and roots are habitats for a wide range of microbes that easily degrade toxic pollutants, which improves the treatment process. As illustrated in Figure 3, plants use various processes to absorb organic and inorganic pollutants, including phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizofiltration. These mechanisms constitute the basis of phytoremediation technology.

3.2.1 Phytoextraction

Phytoextraction is a process in which radioactive nuclide concentrates in the shoots of the plants. The radionuclides are transferred from the roots to the shoots through the vascular bundles, forming a complex with the biomass of the shoots, converting the radionuclides to a less toxic form^[66,67]. The benefit of this process is the easy removal of radionuclides without disturbing the soil structure and its fertility. In the **chernobyl exclusion zone**, the most abundant radioactive nuclide is frequently concentrate by **common heather** or **amaranthus** species.

3.2.2 Rhizofiltration

In rhizofiltration, radionuclides are adsorbed and

Table 4. Various Mechanisms of Radionuclide Microbial Bioremediation^[53]

Mechanism	Radionuclide	Microbes	Ref.
Metabolism-dependent; Sulphide precipitation Transport Intracellular compartmentation Sequestration by proteins, peptides Immobilization by chemical reduction Siderophore complexation	U ⁶⁺ , Ga ⁺³ , Cr ⁺³ , Pu ⁺⁴ , Sc ⁺ , In, Ni, U Th	<i>Shewanella putrefaciens</i> <i>Desulfovibrio desulfuricans</i> <i>Desulfotomaculum</i> sp. <i>Geobacter metallireducens</i> <i>Aspegillus niger</i>	[54]
Enzymatic; Direct Indirect	U ⁶⁺	<i>G. sulfurreducens</i> , <i>Clostridium</i> sp., <i>S. putrefaciens</i> , <i>Desulfovibrio vulgaris</i> , <i>D.</i> <i>Desulfuricans</i>	[37,50,51,54-58]
Biomineralization	Cr ⁶⁺ , As sulphides, phosphates	<i>Citrobacter</i> , <i>Acinetobacter</i> <i>johnsonii</i> , <i>Klebsiella planticola</i> , <i>Pseudomonas aeruginosa</i> , <i>P.</i> <i>putida</i> , <i>Salmonella enterica</i> , <i>Escherichia coli</i>	[50-51]
Biostimulation Bioaugmentation Dissimilatory reduction	Pu ⁶⁺ , U ⁶⁺ , Np ⁺⁵ , Tc ⁺⁷ Hg ⁺² , As ⁺⁵ , Fe ⁺³ , U ⁶⁺ , Co ⁺³ , Cr ⁶⁺ Tc ⁺⁷	<i>Geobacter</i> , <i>Arthrobacter ilicis</i> <i>Streptomyces</i> , <i>Aciditobacillus</i> <i>ferrooxidans</i> , <i>P. putida</i> , <i>Ralstonia</i> <i>eutropha</i> , <i>Pyrobaculum</i> <i>islandicum</i> , <i>Geobacter</i> , <i>Sulfospirillum barnesii</i> , <i>Rhodanobacter</i> sp., <i>Desulfuromusa ferrireducens</i> , <i>Deinococcus radiodurans</i> , <i>Sulfurospirillum barnesii</i>	
Biotransformation Biosorption Bioaccumulation	Pb ⁺² , Zn ⁺² , Cd ⁺² , Sr ⁺² , Th, U ⁶⁺	<i>Pseudomonas mendocina</i> , <i>Bacillus subtilis</i> , <i>Arthrobacter</i> spp., <i>Rhizopus arrhizus</i> , <i>A.</i> <i>nicotianae</i> , <i>Micrococcus luteus</i> , <i>B. vallismortis</i>	[39,43,50,59-62]
Genetically modified microorganisms	Uranyl nitrate, Co, Hg ⁺²	<i>D. radiodurans</i> R1, <i>Escherichia</i> <i>coli</i> , <i>R. eutropha</i> ,.	[42,51,55,63-65]
Bioprecipitation	Uranyl phosphate	<i>D. radiodurans</i>	[60]

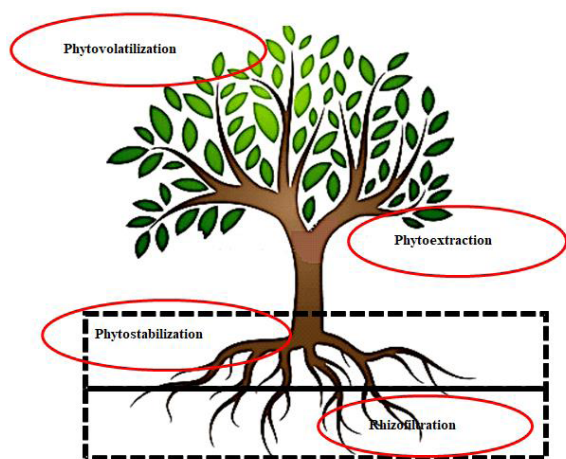


Figure 3. Various process involves in plant remediation of radionuclides^[52].

precipitated in the roots of plants, but their effectiveness depends entirely on the pH^[66,68]. Cesium-137 and Strontium-90 both are significantly adsorbed in the roots of some aquatic plants and algae species such as *Cladophora* and *Elodea*. At present, a large number of

ponds are synthetically designed for this process, in which different aquatic plants and algae are grown to remove radionuclides^[69]. Sunflower is the most efficient plant used for rhizofiltration because it can adsorb up to 95% radioactive nuclide from waste stream within two days.

3.2.3 Phytovolatilization

Phytovolatilization is a process involving the volatilization of radioactive nuclide in the form of less toxic substance^[66,70]. Phytovolatilization process proceeds with transpiration of radioactive nuclide into atmosphere. Radionuclides are not removed during this process, but the process effectively releases radionuclides in the form of volatile substances that are less toxic to the environment. The process is cost-effective compared to other bioremediation processes, and its main advantage lies in its non-disruption of soil structure and fertility.

3.2.4 Phytostabilization

During phytostabilization, the roots of plants are involved in the immobilization of radioactive nuclide^[66]. The basic mechanism of the plant stabilization process is the adsorption and precipitation of radionuclides in the

roots of plants^[71]. The main benefit of this process is that radioactive nuclides are immobilized in roots and can be easily removed if the waste stream of radioactive nuclide is not affected by leaching and soil erosion. Nevertheless, the excessive use of fertilizer in the soil for the restoration of effected area may compromise the benefits of this process^[67]. Table 5 summarized the mechanism of phytoremediation of radionuclide^[67-99]. Recently, the development of phytoremediation technology has received extensive attention and it is expected that it will eventually occupy an important place in environmental remediation. It is envisaged that phytoremediation of radioactive waste will be an essential component of its environmental management and radioactive waste risk reduction.

3.3 Fungal Remediation

Fungal bioremediation, called myco-remediation, is considered more effective for radioactive nuclide removal from waste streams when compared with bacteria fungi^[100,101]. The basic mechanism of myco-remediation is identical to biosorption. The cell wall of fungi is negatively charged, and positive radioactive nuclide form complex on its surface^[102,103]. Some common species of fungi that involves in biosorption are *Xerocomus*, *Cladosporium Paecilomyces*, and *Penicillium*, which effectively adsorb radioactive nuclide such as Promethium-239 and Americium-241 because these fungal species use the radioactive energy of these nuclide for their growth^[104]. Table 6 summarized radionuclides fungal remediation^[105-110]. The advantage of using fungi for remediation is that they are natural decomposers and secrete enzymes that dissolve toxic contaminants without any hazardous effects. This approach allows the development of an eco-friendly way to treat and extract/recover precious radionuclides.

4 BIOREMEDIATION OF ACTINIDES AND THEIR FISSION PRODUCTS

The second series of f-block elements with valance shell electronic configuration $[Rn] 5f^{1-14}, 6d^{0-10}, 7s^2$ are known as Actinides^[111]. All elements in the actinide series are radioactive in nature, and radioactive decay releases a significant amount of energy. The radioactive decay of various actinides is depicted in Figure 4. The most abundant and naturally occurring actinides on Earth are uranium and thorium, while plutonium and others are artificially synthesized. Exposure to these radionuclides is a concern for health and ecology. In biotechnological applications, microbes possess a massive capability for undergoing sustainable manipulations and are, therefore, used for excretion of radionuclides from environment. Radionuclides are non-destroyable but transformable^[50]. Bioremediation of various actinides is discussed as follows.

In nature, about 99% of uranium exists as Uranium-238 with a half time of 4.47×10^9 years^[111,112]. Uranium-238 is an alpha particles emitter radioactive isotope abundantly

found in nuclear wastes including nuclear weapon generation and recycling as well as during nuclear fuel production. There are several microorganism that shows strong affinity to remove uranium from aqueous solutions by using some enzymes involving a reduction process that converts Uranium +6 soluble species into Uranium +4 insoluble species via precipitation of Uranium +4 with the use of some phosphate ligands or by forming complex with the surface of cell, known as biosorption^[113,114]. The most common microbes, including bacteria, are *Geobacter metallireducens* ferric reducing bacteria, *Shewanella oneidensis*^[115,116], *Clostridium*^[21], *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* that show affinity with uranium and can remove it through complexolysis, redoxolysis, bioprecipitation and biosorption. There are two classes of microorganisms that have affinity with uranium. The first class consists of bacteria that conserve energy during reduction of Uranium +6 to Uranium +4 for their anaerobic UO_2^{2+} growth, and the second class consists of bacteria without the ability to store energy for their metabolism during reduction. *Geobacter metallireducens* and *Shewanella oneidensis* are most common example of bacteria that conserve energy for their growth during reduction process^[117]. On the other hand, *Clostridium*, *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* are common example of bacteria that cannot store energy for their metabolism during reduction of radioactive nuclides^[114,118,119]. The basic mechanism of all bacteria is the presence of several types of cytochromes on the cell surface, which are involved in the reduction of uranium and act as electron donors^[120,121]. Figure 5 illustrates well the reduction of Uranium +6 to Uranium +4 through interaction with bacteria.

Neptunium is the fourth element in the actinide series after uranium. Neptunium is highly radioactive nuclide emitting alpha radiations with a long half-life about 214×10^6 years, and human exposure to it will cause serious health issues^[122,123]. Currently, neptunium is majorly produced due to anthropogenic activities including nuclear fuel explosion and nuclear weapons. The most common oxidation state of neptunium is Neptunium +4 and Neptunium +5. Neptunium +4 is an insoluble species that readily precipitates as hydroxide and carbonates, while neptunium +5 is soluble species as it combines with oxygen and form a soluble species, neptunium oxide, that is abundantly found in aquatic media^[124-126]. Several classes of microbes have affinity with neptunium including marine algae and bacteria. *Pseudomonas aeruginosa*, *Streptomycesvirido chromogenes*, *Scenedesmus obliquus* and *Micrococcus* are some common marine algae that involve the removal of neptunium by forming complex on their cell surface. *Pseudomonas fluorescens* is well-known marine algae that efficiently adsorb neptunium +5 from aqueous media at neutral pH by forming complexes on its surface^[127]. Similarly, some bacteria are also known for the

Table 5. Various Mechanisms of Radionuclide Phytoremediation^[66]

Mechanism	Radionuclide	Plant species	Ref.
Phytoextraction	⁹⁹ Tc	Deciduous forest, Triticum, Lolium, Mixed grass species	[72-74]
Phytoextraction	⁹⁰ Sr	Pinus Radiata pine, ponderosa pine, Vitis, Agrostis, Sorghum, Panicum, Paspalum Mycorrhizal association, Salix (willow), Calotropis, Vetiveria, Chromolaena, Cannabis	[75-83]
Rhizofiltration	⁹⁰ Sr	Catharanthus, Helianthus, Brassica, Eichhornia,	[84-87]
Phytoextraction	⁹⁰ Co	Melilotus, Sorghum, Trifolium	[88]
Phytoextraction	²²² Rn	Helianthus, Festuca, Zea	[89]
Phytoextraction	²²⁶ Ra	Helianthus, Festuca, Zea	[89]
Phytoextraction	^{238,235} U	Helianthus, Brassica, Phaseolus, Pisum, Beta, Brassica, Amaranthus	[84,90,91]
Rhizofiltration	^{238,235} U	Brassica, Chenopodium, Eichhornia	[84,92]
Rhizofiltration	^{239,240} Pu	Sargassum, Hordeum, Soya	[93,94]
Rhizofiltration	²³⁷ Np	Triticum, Lolium	[95]
Phytoextraction	²¹⁰ Po	Tabaccum (tobacco)	[96]
Phytoextraction	¹³⁷ Cs	Brassica, Spinacia, Lactuca, Raphanus Salix (willow), Calotropis, Vetiveria Vetiveria, Soya, Pisum, Avena Mycorrhizal association	[78,79,81,97,98]
Rhizofiltration	¹³⁷ Cs	Helianthus, Eichhornia, Catharanthus	[84-86,99]

Table 6. Various Mechanisms of Radionuclide Myco-remediation^[105]

Mechanism	Radionuclides	Fungi	Ref.
Biosorption	²³³ U	Rhizopus arrhizus, Gibberella fujikuroi, G. fujikuroi NCIM 665, G. saubinetti NCIM 851, richoderma harzianum, Rhodotorula glutinis	[106,107]
Biosorption	²³⁹ Pu	Rhizopus arrhizus, Gibberella fujikuroi, G. fujikuroi NCIM 665, and G. saubinetti NCIM 851	[106]
Biosorption	²⁴¹ Am	Rhizopus arrhizus	[108]
Biosorption	¹⁴⁴ Ce	Rhizopus arrhizus, Gibberella fujikuroi, G. fujikuroi NCIM 665, and G. saubinetti NCIM 851	[106]
Biosorption	¹⁴⁷ Pm	Rhizopus arrhizus, Gibberella fujikuroi, G. fujikuroi NCIM 665, and G. saubinetti NCIM 851	[106]
Biosorption	^{152,154} Eu	Rhizopus arrhizus, Gibberella fujikuroi, G. fujikuroi NCIM 665, and G. saubinetti NCIM 851	[106]
Bioaccumulation	^{152,154} Eu	Saccharomyces cerevisiae	[109]
Biosorption	¹³⁷ Cs	Ladosporium, cladosporoides	[110]
Biosorption	⁹⁰ Sr	Ladosporium, cladosporoides	[110]

removal of soluble oxide species from aqueous media to insoluble species neptunium +4 easily by using enzymes and complexing activities. *Shewenella putrefaciens*, *Citrobacter species*, *Desulfovibrio desulfurains* are some common bacteria that reduce neptunium +5 to neptunium +4 and they easily precipitate out as neptunium phosphate in the presence of phosphates.

Plutonium is another trans-uranium artificial element. Plutonium-238 is considered a highly radioactive isotope emitting beta radiations and has a half-life of 87.7 years. Plutonium is considered most debatable actinide element due to its dual uses in disrupting nuclear weapon and nuclear energy generation, which accounts for its large presence in the nuclear waste stream. The most common oxidation state of plutonium is +4, and plutonium +3, +5 and +6 are also stable species. Radioactive decay series of Plutonium-238 is described in Figure 4. Limited data is available on microbial interaction with plutonium. Microbial interaction with plutonium and its reduction from

plutonium +4 to plutonium +3 has been reported by using ferric reducing bacteria, but re-oxidation of plutonium +3 occurs spontaneously. The reduction of plutonium +5 and plutonium +6 to plutonium +4 via enzymes with the aid of bacteria has also been done by using *Shewenella putrefaciens*, *Shewenella oneidensis*^[128]. These bacteria can convert the soluble species of plutonium into insoluble species by mean of reduction and then easily precipitate out with some inorganic complexing ligands; however, it has been reported that oxidation state of plutonium is eventually altered by microbial system and that the solubility of plutonium depends on the redox potential and pH of the solution. A case study investigated the reduction of plutonium from plutonium +4 to plutonium +3 by using microorganisms, in which all electron acceptors and markers were observed, and it revealed that the release of low level of plutonium from deposits with ferric reduction, indicating that plutonium is resistance to reduction mobilization^[129,130]. Americium is also a trans-uranium element. Americium-241 emits alpha particles and decays into Neptunium-237 with

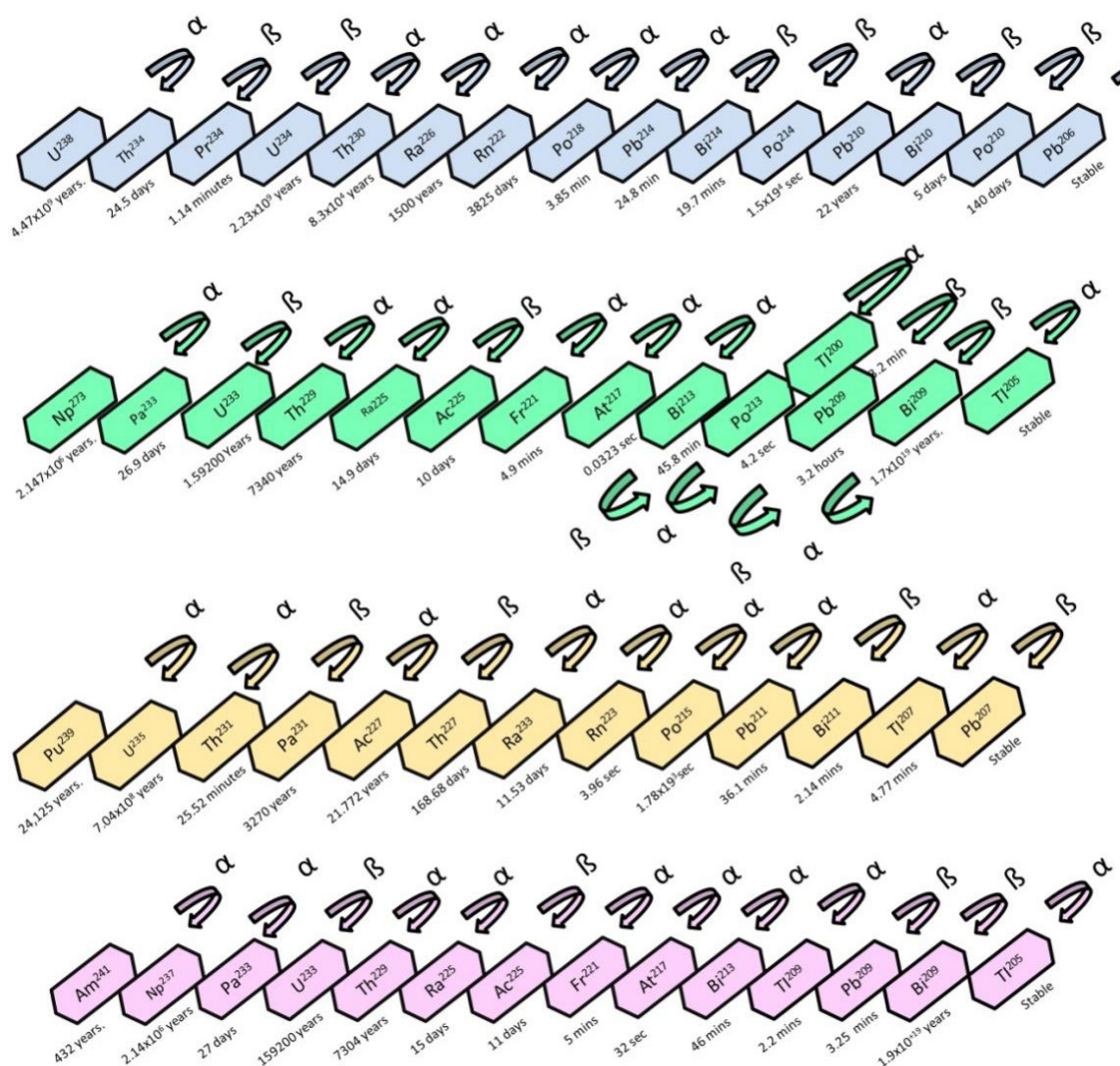


Figure 4. Radioactive decay series of various actinides Uranium²³⁸, Neptunium²⁷³, Americium²⁴¹ and Plutonium²⁵³[111].

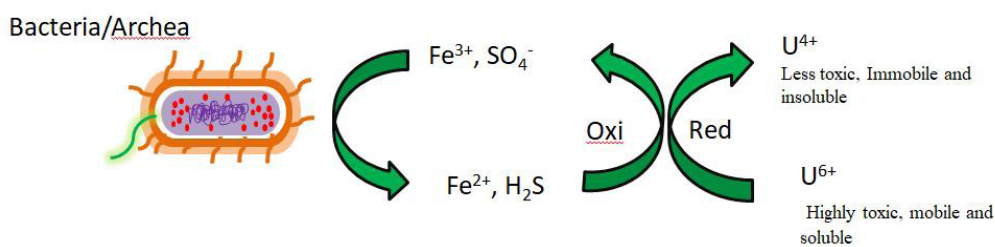


Figure 5. Microbial reduction of uranium[52].

a half-life of 432.2 years. Americium has several oxidation states from +2 to +7 but Americium +2 is usually found in solid state. Several microbes play an important role in interacting with americium, reducing Americium +3 to Americium +2, and adsorbing efficiently on their surface. Some common microbes involved in the reduction from the recovery of americium from devastate include *Escherichia coli*, *Candida utilis*, *Ochrobactrum anthropi*, *Flavobacterium*, *Pseudomonas gladioli* and *Chryseobacterium indologenes*[131], *Rhizopus arrhizus*[106]. The radioactive decay of all these

neptunium, plutonium and americium are described in Figure 4.

Techetium-99 is beta emitter radioactive nuclide with a half-life of 2.13×10^5 years. Technetium-99 is important component of nuclear waste and is highly radioactive[132]. The chemistry of techetium is totally depending upon environmental solubility. The most common oxidation state of is techetium +7, which exists as techetium tetra oxide (TcO_4) and is highly mobile species in environment because

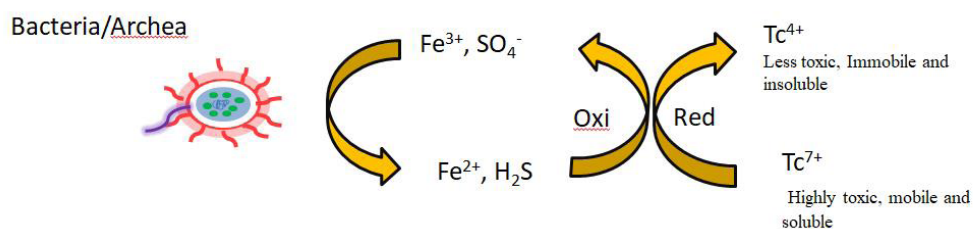


Figure 6. Microbial reduction of Technetium^[52].

it has little adsorption on the cell surface due to its high solubility. However, through the use of some microbes, technetium +7 is converted to technetium +4 by the reduction process and forms insoluble complex technetium dioxide (TcO_2)^[133-135]. Most common examples of microbes that involve in bioreduction of technetium includes *Shewanella putrefaciens*, *Geobacter metallireducens*, *Rhodobacter sphaeroides*, *Pseudomonas denitrificans*, *Pseudomonas species* and *Escherichia coli*^[136,137]. *Escherichia coli* is considered the most suitable for the bioreduction of technetium. Firstly, in anaerobic culture, *Escherichia coli* reduces the technetium +7 to technetium +4 and precipitates it on the surface of the cell^[138]. The use of hydrogenase allows them to form formate hydrogenase complexes^[139]. Furthermore, *Desulfovibrio desulfuricans* and other related strains also use formate as an electron donor to reduce technetium and form insoluble complexes on the surface^[140]. The reduction of technetium +7 to technetium +4 by interaction with bacteria is well illustrated in Figure 6.

Cesium is a radioactive nuclide produced as a fission product. Cesium-137 has a half-life of about 30 years. In the environment, large amounts of cesium are released as a result of nuclear explosions. In the environment, the most common form of cesium is cesium-137 in the Fukushima, Chernobyl and Goiania accidents. Microbes play a key role in the bioaccumulation of cesium, but pre culture studies revealed insufficient uptake of cesium by microorganisms. However, the more efficient uptake of cesium by microorganisms is highly promising^[141]. This is highly similar to the behavior of potassium ions uptake given the similar metabolic transport system of both cations. Most studies on the uptake of cesium ions are shallow. From aqueous media, microorganisms simply adsorb them on their surface by forming complex at alkali pH. Strontium-90 is a fission product that undergoes beta particle emission with a half-life of 29 years. This fission product is formed during exploitation of nuclear reactors. It is frequently emitted as cesium-137 because it is less volatile but is considered the most dangerous radionuclide pollutant. Therefore, its accumulation is necessary because it present in significant amount in nuclear explosion waste stream. Microbes perform a vital role in the removal of radionuclides as they efficiently adsorb strontium ions on

their surface by forming complexes. *Micrococcus leteus* is a common example for strontium adsorption. Strontium binding site is present on the surface of *Micrococcus leteus*, and Strontium ions easily displaced by divalent ion and chelating agents^[142,143]. Radium has the highly stable isotope Radium-226 with a half-life of 1600 years. Radium is usually associated with uranium ore and also dissolved with uranium at the uranium milling site, so it can be precipitated by using barium sulfate and barium chloride to form a radioactive barium sulfate/radium sludge. Its supernatant is released into the environment and contains some radionuclide of radium. Microbes are important to remove radium-226 by co-precipitation with other metallic ions through reduction. *Actinobacteria throbacter*, is a well-known species that remove mobile manganese and Radium +2 by co-precipitation, while bacteria reduce Manganese +4 to Manganese +2^[58,142].

5 EXISTING RADIOACTIVE NUCLIDE WASTE MANAGEMENT PROGRAMS AND FUTURE STRATEGIES IN DEVELOPING COUNTRIES

A program of action or plan designed by a government or different organizations to influence decisions is called a policy. At the beginning of the nuclear era, countries that started introducing nuclear energy were ignorant about nuclear waste and its remediation. To deal with destruction of radionuclides, most of the countries are developed and urbanized and they have adopted basic strategies and methods for the disposal of nuclear waste. Some countries disposed the radioactive nuclide on site storage without proper management for their disposal at national level. In 1995, International Atomic Energy Agency, published the basic principle for proper disposal of radionuclide waste and its ethical issues to reduce the hazardous effects of radioactive nuclide on human health and the environment.

According to this statement, radioactive nuclide waste should be properly managed to minimize the risk to human health and provide an appropriate level of environmental protection. This management practice also ensures that radioactive nuclide waste had little effect on the next generation. Radioactive nuclide waste should be handled within proper and legal national framework, including independency of regulatory function and clear allocation of responsibilities. All the major steps in production and the

management policy radionuclide waste are interdependent, and the safety facilities of the radionuclide waste management plan will be properly established during their life cycle^[144].

The fundamental ideology associated with radionuclide waste disposal can be imposed on all types of radionuclide, despite their different chemical and physical characteristics and origins. Based on these principles, all countries have their rules and regulations as well as their national policies that describe the basic requirements and aims for the legislative and regulatory bodies that includes operative measures and administration^[145]. These fundamental principles explain the situation, state priority, structural, financial, and human resources. In 1922, International atomic energy agency recognized the basic principles and essentials of the advanced policies related to radionuclide waste management. To obtain better policy principles, national waste management plans should be adapted to their implementation in practice and to changing conditions in the country or the world.

The rules for radionuclide waste management, once developed, need to be implemented in practice. Many steps are involved in the implementation. The first is the development of a management strategy, the assignment of strategic responsibilities, and then the acquisition of availability that will help expand the policy. The International Atomic Energy Agency has developed an availability checklist that aids in the development of appropriate strategies for other countries. This checklist includes an assessment of the waste management system, the classification of radionuclides, and an assessment of the sources of radionuclide waste. The second step of the radionuclide waste implementation policy is to identify the endpoints of the waste. Finally, optimal management strategy is developed and responsibilities are assigned for their implementation. There are two options for strategy development: One-stage method and the two-stage methods. The one-stage method is also called nationalized plan that has single waste operator to evaluate the strategy and its implementation. The two-stage method starts with the description of general management policy provisions by an organization such as the government, while in a second step these management policies are implanted by some private organization such as a single company waste operator.

To dispose of all radioactive nuclide waste in developing countries in a safe and sustainable way, National Energy Agency provides considerable assistance. To this end, the National Energy agency has issued strategic management plans, which is the responsibility of the Radionuclide Waste Management Committee (RWMC), to understand the basic problems and challenges faced by all developing countries during the disposal of radioactive nuclides. The recognized designed area of interest integrated the following.

- Development of a proper and sustainable management system that includes financing;
- Disposal of radioactive waste along spent fuel trans-

portation through optimized and robust roadmaps development;

- License must be approved for geological repositories for high and low-level waste;
- Execution of deep geological disposal for industries;
- Decommissioning is effective;
- Long term preservations record and knowledge management and memory;
- Effective executive of all types of radionuclide waste despite of its origin.

6 CONCLUSION

With the latest advances in atomic energy area, radionuclides are generated to meet the demand for nuclear energy and the safe disposal of these radionuclide wastes is a current issue. The release of radioactive nuclide waste from nuclear sites and its spent sources including mining activities, industrial activities, land fillings, agrochemical waste, nuclear weapons recycling, nuclear weapon and nuclear energy generation causes deteriorate effect on environment as well as on human health. These radioactive nuclides majorly consist of high-energy beta and gamma radiation emitters that cause serious health issues such as cancer after excessive exposure to them. Natural resources such as water and soil are also at risk of contamination by radionuclide leaching due to the geological map of mining activities. Hence, to deal with these serious issues, there exists an urgent need to develop nuclear waste management programs that provide an effective route for the disposal of radioactive nuclear waste. Historically, different methods such as solvent extraction, precipitation, ion exchange, and electrochemical process for the effective removal of radio nuclide from waste nuclear stream have been adopted. However, in the recent era, bioremediation demonstrates a great potential to use microorganisms/biomass for the removal and treatment of radioactive nuclide from waste streams, as they are easy to cultivate, economical, and non-chemical pollutants. Therefore, biotechnology plays an important role in different industrial sectors and for environmental protection. In this review, different types of biomass such as bacteria, fungi and plants were reported for the effective treatment and the restoration of radioactive nuclides. All three modes of bioremediation serve as a prime candidate for the management of radioactive waste. Moreover, to dispose of all radioactive nuclide waste in developing countries in a safe and sustainable way, an efficient strategy for radioactive nuclide waste management was also necessitated.

Acknowledgements

Not applicable.

Conflicts of Interest

The author declared no conflict of interest.

Author Contribution

Cheema HA studied, wrote, and reviewed this article.

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